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## Improved image analysis procedures for monitoring activated sludge systems with filamentous bulking

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### Abstract

Activated sludge systems are frequently used in wastewater treatment plant. This process is the most suitable and studied system. However, several problems are being always detected, such as filamentous bulking. Filamentous bulking is typically caused by an overabundance of filamentous organisms that interfere with the settling and compaction process. This phenomenon can be studied and related with settling parameters by automated image analysis using different microscopy acquisitions. However, by using these standard image analysis procedures some relevant information about the state of the sludge is enclosed. Conventional routines, using monochrome images are not suitable to detect the filamentous bacteria which are gram-positive or gram-negative. Moreover, the traditional image acquisition methodologies are not capable to detect both viable and damaged bacteria present within the sludge. Presently, the gram-stain evaluation is performed by visual inspection and manual counting using a microscope which is a tedious procedure. Also, to overcome the lack of viability information, an epifluorescence staining method composed with two nucleic acid-binding stains can be used.

For this study, a lab-scale activated sludge reactor was monitored during 100 days through image analysis information and the operational parameters were modified inducing filamentous bulking. Morphological changes were investigated by using new acquisition methods such as epifluorescence staining LIVE/DEAD® BacLight™ Bacterial Viability Kit, the LIVE BacLight™ Bacterial Gram Stain Kit and the traditional bright field. The overall results revealed an improvement of the sludge morphological characterisation, combining these new image analysis procedures with the conventional routines.